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# Cytogenetic Studies in Lymphocytes of Patients with Rectal Cancer

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**Spontaneous and clastogen-induced chromosomal instability in a high-risk group (i.e., 33 patients with rectal carcinomas) was investigated using peripheral blood lymphocytes as target cells. In addition to the analysis of spontaneous and clastogen-induced chromosome aberrations, this study also included classical karyotype analysis and scoring of sister chromatid exchanges (SCE) in some of the patients. Diepoxybutane (DEB), 4-nitroquinoline-1-oxide (NQO), and bleomycin were used as standard clastogens. Lymphocytes of healthy control individuals were studied in parallel with each cancer patient. While only slight but significant differences could be detected of the average spontaneous, DEB- and bleomycin ($G_2$)-induced chromosome breakage between patient and control lymphocytes, individual patients and two of the control individuals showed a more distinct increase in the frequency of the studied end points. These increases were documented by a variegated mosaicism of karyotypic changes and by an increased breakage rate induced by the clastogens. Neither the bleomycin-exposure in the $G_1$ phase nor SCE was capable of detecting differences between the patients and controls. Of particular interest in the sense of high-risk individuals were seven patients and two control persons whose lymphocytes exhibited increased chromosomal sensitivity under more than one of the studied experimental conditions.**

## Introduction

On the cellular level, the process of malignant transformation is caused by a complex series of genetic changes (mutations). In contrast to previous assumptions, the basic role of these mutational events is not restricted to the so-called initiation phase, but is equally relevant in promotion, transformation, tumor progression, and metastasis (*1–7*). Frequency and order of the mutational events may vary from neoplasia to neoplasia, but their presence is intimately involved in oncogenesis.

Early in the development of tumor cytogenetics, a specific type of human genetic disease attracted the attention of cytogeneticists. Its main features were increased spontaneous and induced chromosomal instability and a striking cancer proneness (*8*). These recessively inherited "chromosomal instability syndromes," therefore, were regarded as model diseases documenting the close relationship of genetic damage and malignant transformation (*9,10*). Patients with those disorders may be regarded as extreme variants of a possibly common interindividual variability of genome stability and, thus, of mutagen susceptibility (*11*).

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About 12 years ago, we published data on chromosomal aberrations found in blood lymphocytes of tumor patients undergoing a cytostatic interval therapy with methyl-CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] fluororacil, and vincristin (*12*). One of the most striking findings of this study was the extreme interindividual variation of chromosomal response to an identical scheme of therapy with exactly comparable doses. With this variability in mind, we appreciated the hypothesis of Hsu (*11*) that a close relation might exist between the genomic instability of an individual and his or her cancer risk. If a variety of mutational events actually plays such an important role in the multistep process of malignant transformation, every factor increasing the number of those events, for instance, a genetically determined individual genomic instability, should also increase the individual cancer risk. And even in those cases not showing an increased spontaneous chromosomal instability, mutational stress eventually could provoke an immanent mutagen susceptibility. If these considerations are valid, cancer patients should be loaded by an increased genomic instability which should express itself not only by an increased spontaneous aberration rate but also by a higher sensitivity versus mutagenic influences than is found in healthy persons of the average population. Several reports demonstrating an increased incidence of karyotypic changes and spontaneous chromosomal breakage in non-neoplastic somatic cells of cancer patients seem to support

this view. As reviewed by Heim et al. (*13*), these findings, however, were not too consistent; several authors did not find any increase. In addition, many of these studies were deficient in analyses of control groups and did not consider mutagen sensitivity.

Having been involved for many years in studies on the cytogenetics of human solid tumors, we took the opportunity to obtain peripheral blood lymphocytes from a group of patients with rectal tumors and to analyze their chromosomal stability and mutagen sensitivity using various techniques and end points: In addition to karyotyping and determining the spontaneous frequency of breakage and sister chromatid exchange (SCE), the sensitivity of these cells to diepoxybutane (DEB), 4-nitroquinoline-1-oxide (NQO), and in a number of cases also to bleomycin was studied using chromosomal breakage and SCE as end points.

## Patients and Methods

Cultures of peripheral blood lymphocytes obtained from 33 patients with colorectal (in 32 cases rectal) cancer were the basis of this study (Table 1). Details of the cytogenetic and oncogenetic data of the tumors have been published elsewhere (*14*). In addition, control cultures were set up in parallel from peripheral blood of 28 healthy individuals selected by age and sex for controls.

In a first pilot study, karyotype analysis using standard GTG banding was performed in 15 of these patients as well as in a comparable control group of healthy individuals. Spontaneous chromosome aberrations were analyzed from 100 well-spread metaphases prepared from bromodeoxyuridine (BrdU)-labeled cultures of 28 patients and 28 control individuals. In addition, chromosomal sensitivity to chemical mutagens/carcinogens was studied using a

**Table 1. Data for the patients included in this study.**

| | | | Characterization of tumor | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Staging | | | | |
| Code no. | Age, years | Sex | Localization | Grade of malignancy | pT | pN | pM | Cytogenetics abnormal[a] | Lymphocyte analyses[b] |
| 57 | 57 | M | I | 2 | 3 | 0 | 1 | ND | All |
| 58 | 72 | F | I | 1 | 4 | 3 | 1 | ND | All |
| 59 | 44 | F | L | 2 | 2 | 0 | 0 | V | All |
| 64 | 52 | M | I | 2 | 4 | 2 | 1 | ND | All |
| 66 | 46 | M | L | 3 | 3 | 2 | 0 | ND | All – BLM |
| 67 | 58 | M | L | 2 | 3 | 1 | 0 | ND | SS, DEB |
| 69 | 60 | M | L | 3 | 4 | 3 | 1 | ND | All |
| 71 | 72 | F | I | 2 | 3 | 2 | 0 | II | SS, IS |
| 72 | 57 | M | L | 2 | 2 | 1 | 0 | III | SS, IS |
| 73 | 55 | M | L | 2 | 3 | 1 | 1 | II | K |
| 74 | 65 | M | I | 3 | 3 | 3 | 0 | V | All – BLM |
| 76 | 56 | M | L | 2 | 4 | 3 | 1 | ND | K |
| 77 | 58 | M | L | 2 | 3 | 0 | 0 | IV | K |
| 80 | 60 | M | I | 2 | 3 | 2 | 0 | IV | K |
| 81 | 75 | F | C | 3 | 3 | 3 | 0 | III | All |
| 82 | 51 | M | L | 3 | 2 | 1 | 0 | ND | All |
| 95 | 67 | M | I | 2 | 1 | 0 | 0 | III | K |
| 96 | 55 | M | I | 2 | 3 | 0 | 0 | III | All |
| 113 | 74 | M | I | 2 | 3 | 0 | 0 | IV | SS, IS |
| 114 | 61 | F | I | 3 | 3 | 0 | 0 | V | SS, IS |
| 115 | 75 | M | I | 2 | 3 | 0 | 0 | III | SS, IS |
| 117 | ND | M | L | ND | ND | ND | ND | V | SS, IS |
| 119 | 71 | F | U | 2 | 4 | 0 | 0 | III | SS, IS |
| 121 | 73 | F | I | 2 | 3 | 1 | 0 | V | SS, IS |
| 122 | 52 | F | I | 2 | 3 | 0 | 0 | IV | SS, IS – BLM |
| 124 | 47 | F | I | 3 | 3 | 2 | 0 | V | SS, IS |
| 126 | 63 | M | I | 2 | 3 | 1 | 0 | IV | SS, IS |
| 127 | 59 | F | U | 2 | 2 | 3 | 0 | IV | SS, IS |
| 129 | 70 | M | I | 2 | 3 | 1 | 0 | V | SS, IS |
| 132 | 67 | M | L | 3 | 3 | 2 | 0 | V | SS, IS |
| 133 | 66 | M | I | 2 | 3 | 1 | 0 | III | SS, IS |
| 134 | 50 | M | I | 2 | 3 | 1 | 1 | IV | SS, IS |
| 138 | 47 | F | ND | ND | ND | ND | ND | ND | SS, IS |
| 139 | 65 | M | ND | ND | ND | ND | ND | ND | SS, IS |

Abbreviations: ND, no data available; L, I, U, lower, intermediate, or upper part of the rectum, respectively; pT, pN, pM, classification system of clinical staging of the tumors (*40*) The international staging system of solid tumors defines tumors according to their anatomical extension: pT index for tumor diameter and invasiveness [T1: diameter lower than 2 cm, T2: diameter lower than 5 cm, T3: diameter over 5 cm; T4: infiltration of neighboring tissue]; pN index of affection of regional lymph nodes [N0: no affection, N1: affection of homolateral, N2: affection of bi- and contralateral lymph nodes, N3: fused nodi]; pM index of distant metastases [M1: one distant metastasis, etc.].

[a]Classification system of karyotypic abnormality of the tumor from single abnormalities (I) to extremely complex karyotypes (V), as defined in Rau et al. (*38*).

[b]Type of analysis performed in the present study: "all" means karyotyping + spontaneous breakage + exposure to DEB, NQO, and BLM; K = karyotype only; SS = spontaneous stability; IS = sensitivity to inducing clastogens; – BLM = no bleomycin data.

Table 2. Protocol for testing chromosomal instability.

| Inducing agent | Standard dose | Length of treatment, hr before sampling | BrdU Supplement, hr before sampling[a] |
|---|---|---|---|
| None | – | – | 48 |
| DEB | 0.01 μg/mL | 24 | 24 |
| NQO | $10^{-5}$ mole/L | 24 | 24 |
| BLM ($G_1$) | 200 μg/mL | 3 + 21[b] | No |
| BLM ($G_2$) | 100 μg/mL | 2 | No |

Abbreviations: DEB, diepoxybutane; NQO, 4-nitroquinoline-1-oxide; BLM, bleomycin.
[a]Final concentration = $10^{-5}$ M.
[b]3-hr treatment followed by 21-hr interval.

protocol developed in our laboratory for the analysis of chromosomal instability syndromes (Table 2). Diepoxybutane and NQO were applied to lymphocytes of all patients and control individuals; bleomycin was used only in a limited number of experiments in the sense of a pilot study.

At least 100 "first" metaphases per treatment and individual were analyzed for chromosomal aberrations from BrdU-labeled slides. In addition, in a couple of patients and controls, the SCE rate was estimated from at least 30 "second" metaphases. The Wilcoxon test was used to test the differences between paired breakage rates.

## Results

In a first pilot study, karyotype analyses were performed in part of the patients and controls. In the patients' lymphocytes, karyotypic changes were found about twice as frequently as in the control lymphocytes. Hyper- and hyposomy of the X-chromosome were clonally present in the lymphocytes of three of the patients but in none of the controls. Although other numerical changes were not found in controls, the lymphocytes of one of the patients showed clonal monosomies of chromosomes 19, 21, and 22. Among the clonal structural changes, inversions in chromosome 12 and a deletion in the long arm of chromosome 16 were found in clonal form in a few patients. Nonclonal anomalies of other chromosomes were found in the patients' lymphocytes, but less frequently in those of the control individuals.

The spontaneous chromosomal instability was estimated from BrdU-labeled first metaphases of 72-hr lymphocyte cultures of 28 cancer patients and 28 control individuals. The average breakage rate was slightly but significantly ($p<0.01$) increased in the patients' group compared to the controls (Table 3). The values showed a larger variation in the patients, nine of the samples exhibited breakage rates that were distinctly increased over the average level (Fig. 1). The exchange index was also higher in the lymphocytes of the patients (0.19% versus 0.11% in the controls). The average spontaneous SCE frequency did not differ between both groups (Fig. 1; Table 3). The direct comparison of each patient with the respective control individual revealed a higher breakage rate in 18 of the patients and 4 of the controls, while in 6 cases identical rates were found (Table 3). As bleomycin was proposed by others (see above) as a standard clastogen for the detection of an immanent chromosomal instability, a pilot study was initiated on our material using this cytostatic antibiotic. $G_1$- and $G_2$-phase exposure was included in this analysis (Fig. 2*A,B*). In the $G_1$ experiments (see Table 2), lymphocytes of controls, on average, showed a higher breakage rate, and only 2 out of 10 patients showed a higher individual breakage rate than the respective control. It was mainly the three "high responders" among the control individuals who were responsible for the average differences of aberration rates between both groups.

In the $G_2$ experiments, 7 out of 15 patients had higher breakage rates, and the average breakage rate was slightly increased in the patients' group ($p<0.05$). The average exchange index of the patients was about twice that of the controls. However, high responders were detected in both the patient and control groups (Fig. 2*A,B*). As shown in Figure 2*C*, three patients with familial adenomatous polyposis did not have a higher response to bleomycin than the controls studied in parallel.

Similar to the data on the spontaneous breakage reported before, a slight but significant ($p<0.01$) tendency to increased rates of DEB-induced breakage was

Table 3. Comparison between the group of patients and of control individuals of the frequencies of the various end points studied on lymphocyte cultures under different exposure conditions.

| Exposure | Group | $n$ | $N_m$ | % Aberrations metaphase | No. per 100 metaphases G′ | G″ | B′ | B″ | E | Breaks/ metaphase | Patients vs. controls[a] + | = | – | SCE/metaphase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Untreated | Patients | 28 | 3160 | 5.9 | 2.56 | 0.35 | 2.27 | 1.29 | 0.19 | 0.042 | 18 | 6 | 4 | 5.20 |
| | Controls | 28 | 2980 | 5.0 | 2.65 | 0.53 | 1.47 | 0.79 | 0.11 | 0.026 | 4 | 6 | 18 | 5.05 |
| *BLM ($G_1$)* | *Patients* | *10* | *1180* | *41.8* | *13.98* | *1.61* | *33.63* | *29.07* | *1.21* | *1.00* | 2 | *0* | 8 | – |
| | Controls | 10 | 1020 | 56.3 | 23.14 | 2.45 | 55.39 | 58.23 | 1.74 | 1.53 | 8 | 0 | 2 | – |
| DEB | Patients | 28 | 2940 | 9.4 | 4.28 | 0.78 | 4.25 | 2.14 | 0.41 | 0.074 | 17 | 6 | 5 | 12.93 |
| | Controls | 28 | 2890 | 7.7 | 3.19 | 0.49 | 3.61 | 1.32 | 0.17 | 0.052 | 5 | 6 | 17 | 11.82 |
| NQO | Patients | 27 | 2830 | 14.4 | 5.3 | 1.38 | 9.15 | 5.05 | 2.97 | 0.20 | 14 | 0 | 13 | 20.84 |
| | Controls | 28 | 2900 | 14.3 | 4.3 | 1.17 | 7.21 | 3.79 | 3.14 | 0.18 | 13 | 0 | 14 | 20.23 |

Abbreviations: $N_m$, total number of analyzed metaphases; G′, chromatid gaps; G″, isochromatid gaps, B′, chromatid breaks; B″, isochromatid breaks; E, exchanges; SCE, sister chromatid exchange; BLM, bleomycin; DEB, diepoxybutane; NQO, 4-nitroquinoline-1-oxide.
[a]*Comparison of breakage rates of individual patients and their parallel control:* (+) *breakage rate higher than,* (=) *breakage rate identical to,* (–) breakage rate lower than control.

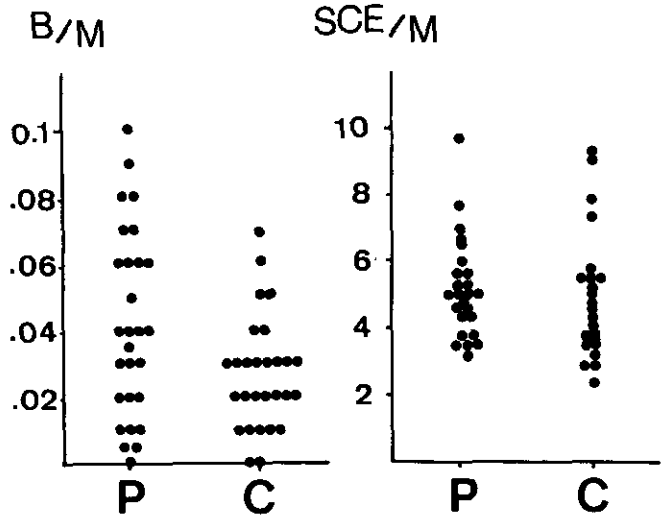


FIGURE 1. Individual spontaneous breakage (left) and SCE rates (right) per metaphase in cultured blood lymphocytes of patients with rectal carcinomas and healthy controls.

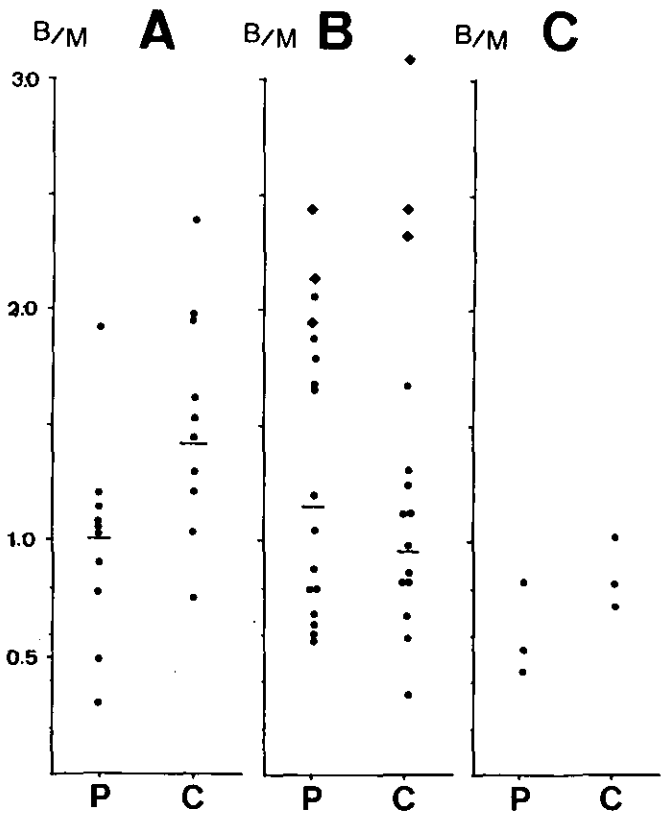


FIGURE 2. Bleomycin-induced breakage rates in cultured blood lymphocytes from patients with rectal carcinomas (P) and from healthy control individuals (C) after exposure in $G_1$ (*A*) and $G_2$ (*B*). (*C*) breakage rates for 3 patients with familial adenomatous polyposis. For experimental protocols see Table 2.

observed in the patients compared to controls. The SCE rates, however, did not differ significantly between patients' and controls' lymphocytes (Table 3). The lymphocytes of 17 patients exhibited a higher breakage rate than those of the parallel controls, while, vice versa, this was valid for only 5 of the 28 controls. But only a few individuals were characterized by a breakage rate more distinctly increased above average (Fig. 3). In addition, the average exchange index in the patients was more than twice that of the controls (0.41% versus 0.17%). The addition of NQO could not detect a significant difference between patients' and controls as a group in regard to average breakage rate ($p > 0.05$) or in regard to the SCE frequency induced by this carcinogen (Table 3). Seven of the patients and three of the controls, however, showed a high susceptibility versus the chromosome-damaging effect of NQO (Fig. 4). As two of the controls were loaded with a high number of NQO-induced interchanges, the average exchange index did not differ significantly between both groups.

A comparison of the data obtained from the various exposure groups defined the chromosomes of seven of the patients and two of the control individuals as being increasingly sensitive under more than one treatment condition (Table 4). In three patients, all, spontaneous, DEB-induced, and NQO-induced chromosomal breakage were increased; two of these patients, in addition, showed a slightly increased bleomycin sensitivity. In three other cases, the elevated spontaneous aberration rate was associated with sensitivity versus NQO. Interestingly, one patient and one control individual, whose spontaneous lymphocyte aberration rate was inconspicuous, proved to be sensitive to both DEB and NQO.

## Discussion

In accordance with several previous reports (*15–23*), a slight spontaneous chromosomal instability and a slightly increased chromosomal sensitivity to clastogens/carcinogens could be detected in a group of patients with rectal carcinoma. As these differences in the average breakage rates in all categories were strongly caused by a few "high responders," we do not believe that it would be justified to regard increased chromosomal instability as a feature of the whole group of patients with rectal cancer.

In the $G_1$ experiments with bleomycin, even more control individuals than cancer patients proved to be sensitive to the clastogenic action of this cytostatic drug. The latter data, however, also should not be overestimated, as only rather small numbers of patients and controls were included in this pilot study on bleomycin.

There was, however, a series of investigations reported that did not find an increased chromosomal instability in cancer patients (*15,24–28*). For the reasons pointed out above, these observations also cannot be judged to absolutely disagree with our findings, as the majority of patients with rectal cancer, indeed, was not burdened with increased chromosomal instability or sensitivity to clastogens. Reports on increased SCE frequencies in blood lymphocytes of cancer patients (*29–33*), however, could not be confirmed by the present investigation.

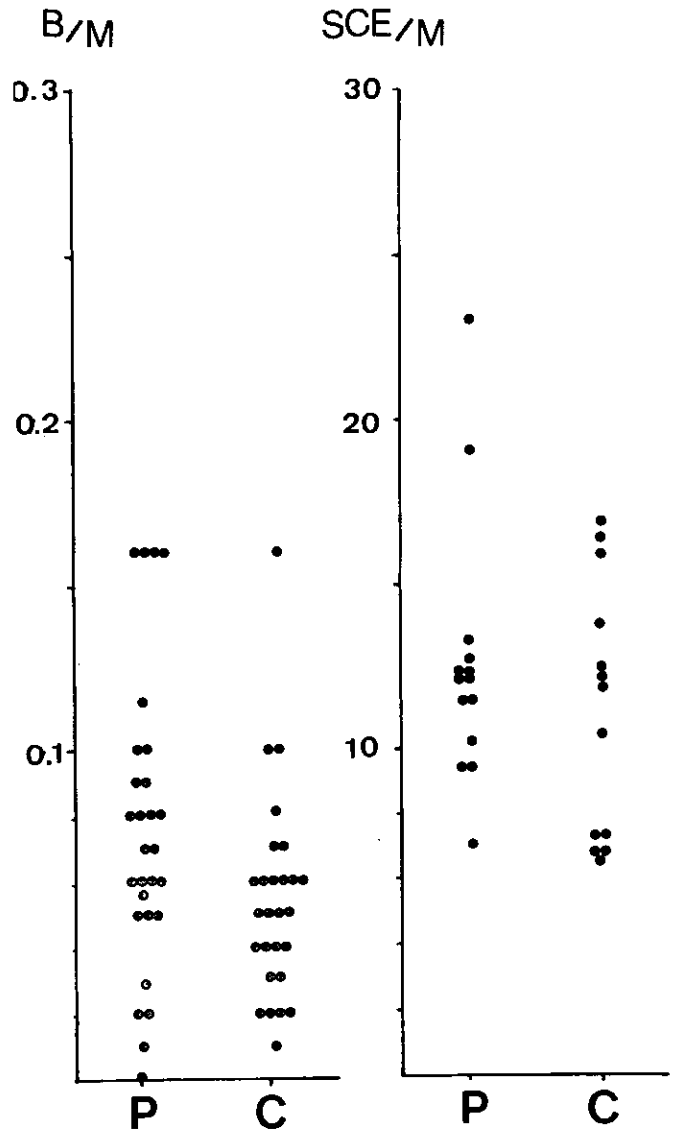


FIGURE 3. Diepoxybutane-induced breakage (left) and sister chromatid exchange rates (right) in cultured lymphocytes from patients with rectal carcinomas (P) and from healthy control individuals (C).

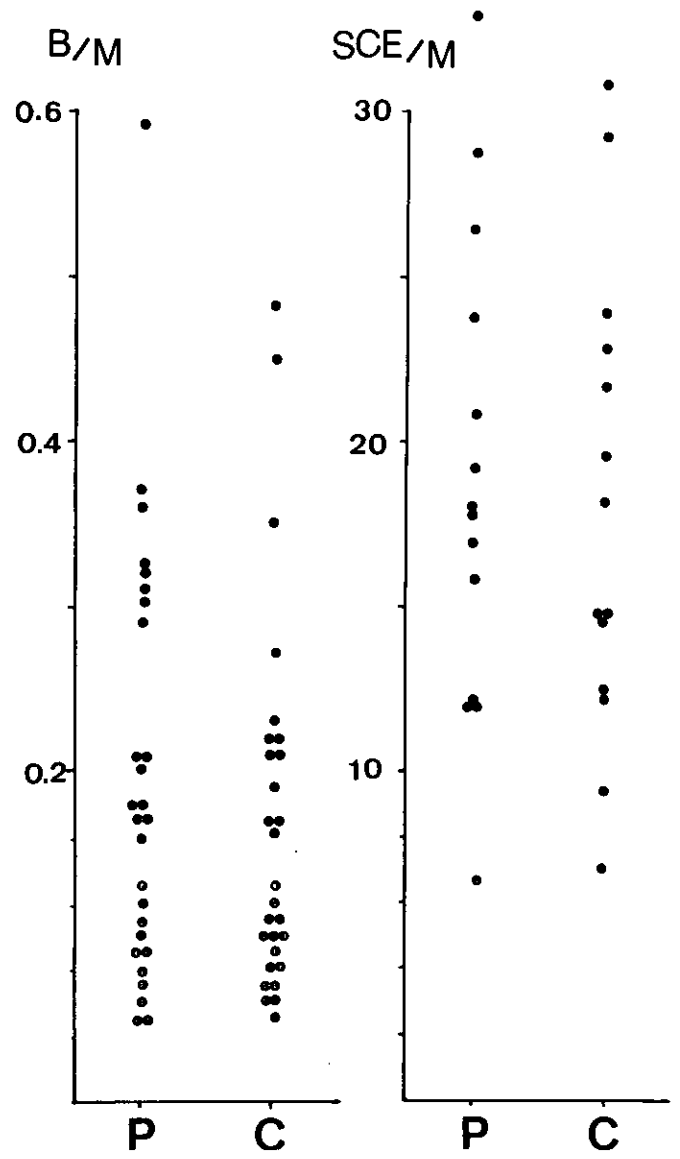


FIGURE 4. 4-Nitroquinoline-1-oxide-induced breakage (left) and sister chromatid exchange rates (right) in cultured lymphocytes from patients with rectal carcinomas (P) and from healthy control individuals (C).

**Table 4. Individuals with increased chromosomal instability under more than one exposure condition.**[a]

| Code no. | Increased breakage in: Untreated lymphocytes | DEB-exposed lymphocytes | NQO-exposed lymphocytes | BLM-exposed lymphocytes |
|---|---|---|---|---|
| 64 | − | + | ++ | − |
| 72 | (+) | − | (+) | + |
| 117 | ++ | − | (+) | − |
| 121 | + | + | ++ | − |
| 122 | ++ | − | ++ | ND |
| 127 | ++ | + | ++ | ++ |
| 132 | ++ | − | ++ | − |
| C113 | + | ++ | − | ND |
| C124 | − | + | ++ | − |

[a] ++ More than twice the average breakage rate; +, more than 1.5 times up to twice the average breakage rate; (+) 1.5 times or nearly 1.5 times the average breakage rate; −, not distinctly increased above the average breakage rate; ND, no data.

These differences in outcomes of various investigations may be due to a variety of factors. A critical review of the respective literature (*13,34*) shows a striking lack of methodological consistency in many of the reports. We agree with Heim et al. (*13*) that only those investigations should be regarded as informative in which "appropriate control populations have been included, and in which the comparisons have been made between groups that are large enough for statistical analysis to be meaningful." But also regarding these basic guidelines, differences of outcome were evident. The studied cell type, therefore, must be regarded as another reason for confounding results. As most studies on somatic chromosomal instability were performed on lymphocytes or, in a few cases on fibroblasts,

and the neoplasms were carcinomas in most cases, the use of inappropriate and therefore uninformative cells might also explain some of these discrepancies.

Another important aspect may be the varying interaction of genetic and environmental factors in the type of respective cancer studied. Hsu (*35*), for instance, had shown that, in patients with cancers of the colon or of the lung the bleomycin-induced chromosomal instability in noncancerous somatic cells was more distinctly expressed than it was in breast cancer patients. In addition, cancer risk must not necessarily be determined by the predisposing genetic load of the whole organism, but could rather be due to a more local tissue-specific chromosomal instability (repair incapability). Therefore, as shown above, analyses restricted to one specific tissue, for instance, to lymphocytes of patients with cancers of other sites and tissues, may lead to false conclusions. It is, therefore, a welcome change that those cell systems derived from the most common cancers are used more and more in experimental mutagenicity testing (*36,37*).

Irrespective of these aspects, from a more general view, testing chromosomal breakage in peripheral blood lymphocytes of individuals exposed to environmental toxins retains its importance as a monitoring system in genetic toxicology because it nevertheless allows detection of "high-risk individuals."

In the present study, as well as in several others, the average aberration rates were strongly determined by a group of high responders. Some of the individuals of this present investigation, in addition, showed chromosomal instability evident under various exposure conditions. These multiple responders, nevertheless, must be regarded as belonging to a high-risk group and are possibly cancer prone. The analysis of the cytogenetic findings in tumors from multiple responders reveals that five of the six multiple responders, for whom respective data were available and two of the high responders in one experiment belonged to the category with the highest level of karyotypic anomalies in the tumor cells (*14,38*).

Therefore, apart from being generally suited to detect predisposition to cancer, cytogenetic screening evidently can detect high-risk individuals. Screening needs to be further investigated by prospective studies (*39*) to determine if correlation or association actually exists between increased chromosomal instability or mutagen sensitivity and human disease, in particular, cancer.

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